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Journal of Chemical and Pharmaceutical Research, 2015, 7(4):1246-1253

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Diversity of some endophytic fungi associated with rice black bug *Paraeucosmetus pallicornis* on rice plant

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ABSTRACT

A new rice insect pest was sighted in some rice producing areas of South Sulawesi Province, Indonesia. This pest is rice black bugs Paraeucosmetus pallicornis. The research aimed to isolation of fungi associated with rice black bugs Paraeucosmetus pallicornis, so as to know the cause of a bitter taste to the rice. The isolation of the fungi consist of three kinds of treatment, namely rice black bugs without sterilization, with sterilization and rice black bugs cut and sterilized. The result showed that the treatment rice black bug without sterilization obtained three genera namely Fusarium sp, Penicillium sp, and Aspergillus sp. In the treatment of rice black bug with sterilization obtained also three genera of fungi, as well as in the treatment of rice black bugs cut and sterilized, respectively Metarizium sp, Aspergillus sp, and Penicillium sp. as son as Trichoderma sp, Penicillium sp, and Aspergillus sp.

Key words: Diversity, *Paraeucosmetus pallicornis*, rice black bugs, Rice plant

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INTRODUCTION

Rice was the staple food in the Indonesian diet, accounting for more than half of the calories in the average diet, and the source of livelihood for about 20 million households, or about 100 million people, in the late 1980s. Rice cultivation covered a total of around 10 million hectares throughout the archipelago, primarily on sawah.

A rice black bug is only as big as a “black bean” but it is very destructive. It sucks the juice from the midrib of leaves and panicles at the milk stage. In most cases, it feeds on the basal part of the tillers most often at night. A report from the Department of Agriculture said that during the vegetative stage, plants attacked by this become stunted. The youngest leaf shoot of the tiller fold longitudinally, turns yellowish to reddish brown, and later dies.

Rice black bugs are common pests to the ears damaged, deflated even empty panicles. Described by some farmers that the rate of moderate-severe attacks can decrease the production reaches 50%. This pest damage by sucking the liquid well on young grain and the grain that is rather old. Grain attacked be deflated / dry and the visible grain leather brown spots. Former the sucking on rice usually issued some sort of liquid sugar and allegedly where the growth of fungi that cause a bitter taste in rice [1] [Figure 1].

The term endophyte was coined by the German scientist, Heinrich Anton De Bary in 1884, and used to define fungi and bacteria occurring inside plant tissues without causing any apparent symptoms in the host [2]. In the last few years fungal endophytes have been detected in hundreds of plants including important agricultural commodities as sengon plant [3], bananas [4], maize [5]; cocoa plant [6] and medicinal plant Buah Makassar *Brucea javanica* [7].

Figure 1. Symptom on Rice by *Paraeucosmetus pallicornis.* Control (left) and Symptom by *Paraeucosmetus pallicornis* (right)

EXPERIMENTAL SECTION

Sample Collection

Rice black bugs taken from Gowa and Luwu region, Province of South Sulawesi, Indonesia. The samples were collected by aseptic procedures and brought to the laboratory of Plant Protection Department, Faculty of Agriculture, Hasanuddin University, Indonesia and processed within 24 hours of collection.

Isolation of Fungi Associated With *Paraeucosmetus pallicornis*

The isolation of the fungi consist of three kinds of treatment, namely rice black bug without sterilization, with sterilization and rice black bugs cut and sterilized. By the treatment of rice black bugs without sterilization done with placed rice black bugs on Petri dishes containing PDA medium was as much as 5 tail. Afterwards, they were incubated in the room temperature for several days. From 3 to 14 days after incubation, the fungi were growing and isolated to pure culture in another PDA. By the treatment of rice bugs sterilization do the same thing, but with Alcohol and Natrium hyphoclorit sterilization. The rice lack bugs taken from the field were washed twice in distilled water then surface sterilized by immersion for 1 minute in 70% (v/v) ethanol, 5 minutes in sodium hypochlorite (2.5 % (v/v) available chlorine) and 30 seconds in 70% (v/v) ethanol and then washed three times in sterilized distilled water for 1 minute each time. After surface sterilization, transferred to plates containing potato dextrose agar (PDA, pH 6.8, containing (g/l): potato 200; dextrose 20; agar 15.), which had been autoclaved for 15 minutes at 121ºC and then aseptically supplemented with 100 mg/ml of chloramphenicol (Pfizer) to suppress bacterial growth. Aliquots from the third wash were plated onto PDA to check that surface sterilization had been effective and they were then incubated at 28ºC. Any fungi present was isolated, purified and then maintained at 4ºC on PDA slopes for further identification. For tentative identification, microscopic slides of each fungi were prepared, examined under light microscope (Olympus, USA) and identified with reference to [8,9].

RESULTS AND DISCUSSION

Treatment of Rice Black Bugs Without Sterilization

The result showed that the treatment rice black bug without sterilization obtained three genera namely *Fusarium* sp, *Penicillium* sp, and sp. (Table 1).

Table 1. Diversity of fungi associated with *Paraeucosmetus pallicornis* By the Treatment of Rice Black Bugs Without Sterilization

No	Genera	Macroscopic characterization on PDA media	Microscopic characterization
1	*Fusarium sp*	The colony of white cotton and fine texture	- It have microconidia - Hyphae have septae - Conidia crescent and septae
2	*Aspergillus sp*	The colony is blackish brown	- Smooth Konidiafor some what upright - Conidia round - At the end of the round-shaped Hyphae
3	*Penicillium sp*	The colony is white	- Finger-shaped conidiophores - There are 2-3 hyphae per branch - Conidia round, slightly oval

Fusarium sp

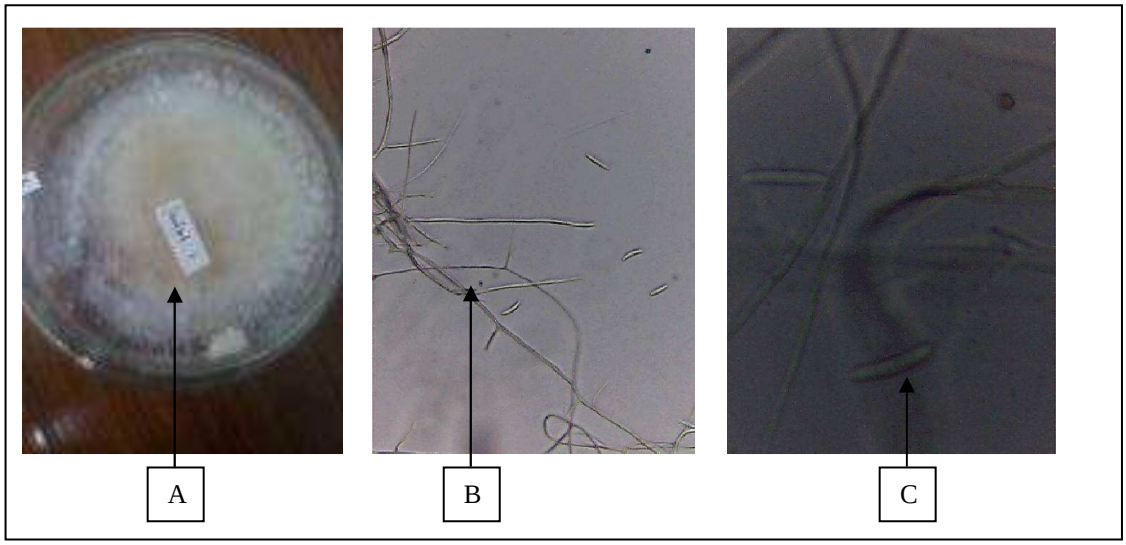

Figure 2. *Fusarium* sp. (a) Colony performance on the PDA (b) hyphae and (c) Conidia

Fusarium sp. characterized by very rapid growth of hyphae and the colony is white cotton and fine texture. Microscopic identification results showed that the crescent-shaped makrokonidia with 3-5 septa and clamidospora is round and slightly oval (Figure 2a, 2b and 2c).

Aspergillus sp

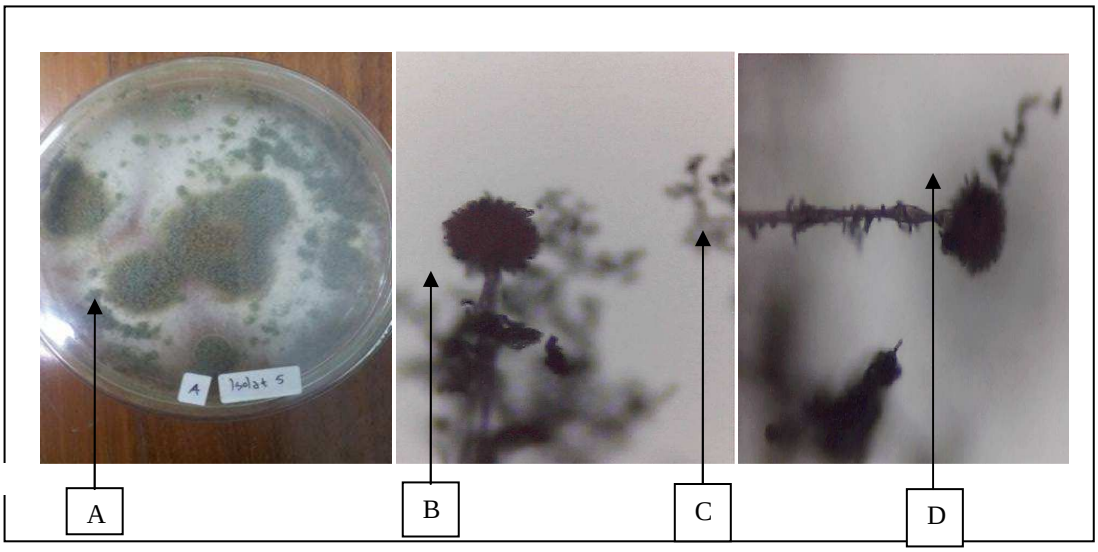

Figure 3. *Aspergillus* sp (a) Colony performance on the PDA (b) Conidia and (c) Hyphae (d) Conidiophore

Aspergillus sp characterized by the colony is blackish brown and white in the margin area, micellium are growing regular with flat colony, thickness and the margin of colony is flat as well. Feature of microscopy: hyphae are asepta, micellium have branches, conidiophores upright. The edge swells and produces vesicle. On the whole vesicle surface micellium are covered, forming phyalide. Conidia are formed subsequently in the phyalide. Conidia are spericle, hyaline, and 1 cell sterigmata (Figure 3a, 3b, 3c and 3d).

Penicillium sp

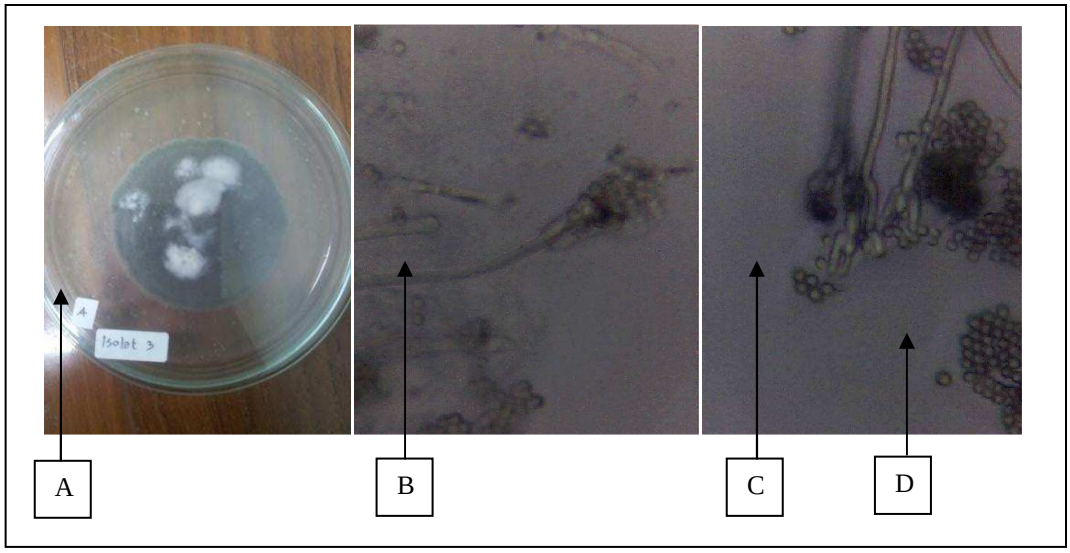

Figure 4. *Penicillium* sp (a) Colony performance on the PDA (b) Conidia and (c) Hyphae (d) Conidiophore
***Penicillium* sp. characterized by the colony is white. Microscopic identification results showed that there are 2-3 hyphae per branch, finger-shaped conidiophores and Conidia round, slightly oval. (Figure 4a, 4b, 4c and 4d)**

Treatment of Rice Black Bugs With Sterilization

The result showed that the treatment rice black bug with sterilization obtained three genera namely *Metarizium sp, Aspergillus sp, and Penicillium sp.*(Table 2).

Table 2. Diversity of fungi associated with *Paraeucosmetus pallicornis* By the Treatment of Rice Black Bugs With Sterilization

No	Genera	Macroscopic characterization on PDA media	Microscopic characterization
1	*Metarrihizium sp*	fungal colonies colored white, then change become dark green with age colonies	- The diameter of mycelium are from 1.98 to 2.97, then arranged with straight, layered and patterned full color one-celled conidia hyaline, cylindrical shape with a size of 9 - Conidiophores are compact like wax composition of phialidae at the ends cylindrical conidia - formed conidia in chains of cells, smooth-walled, colorless and cylindrical "oval"
2	*Aspergillus sp*	The Colony is blackish brown	-Smooth Konidiafor somewhat upright -Conidia round - At the end of the round-shaped hyphae
3	*Penicillium sp*	The Colony is white	-Conidiaphore shaped like fingers -There is a branching hyphae 2-3 - Conidia round, slightly oval

Metarihizium sp

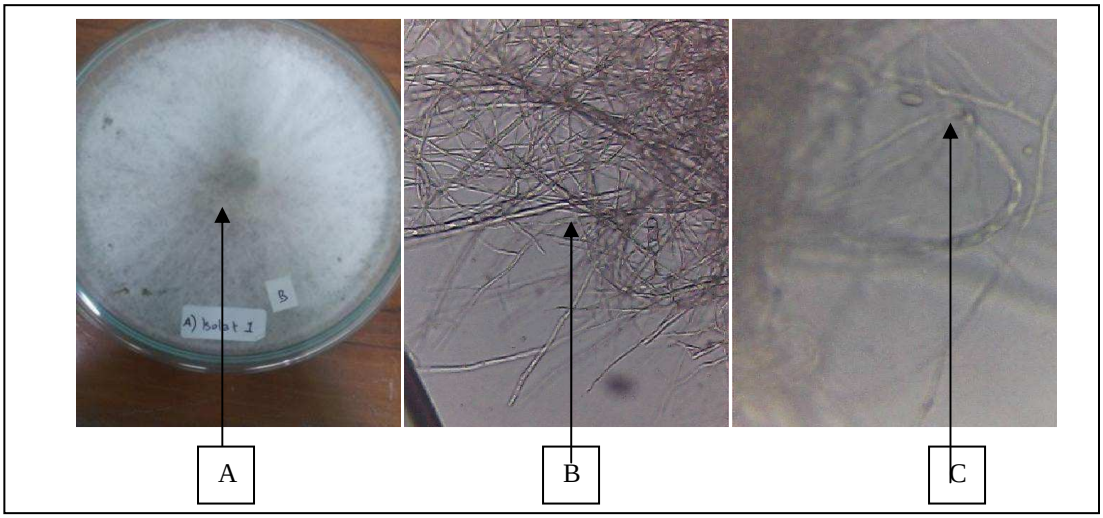

Figure 5. *Metarhizium* sp. (a) Colony performance on the PDA (b) Hyphae and (c) Conidiophore

Macroscopic characterization of *Metarrhizium* sp. on PDA medium are fungal colony colored white, then change become dark green with age colonies. The diameter of mycelium are from 1.98 to 2.97, then arranged with straight, layered and patterned full color one-celled, conidia hyaline, cylindrical shape with a size of 9. Conidiophores are compact like wax composition of phialidae at the ends cylindrical conidia formed conidia in chains of cells, smooth-walled, colorless and cylindrical "oval"

Aspergillus sp

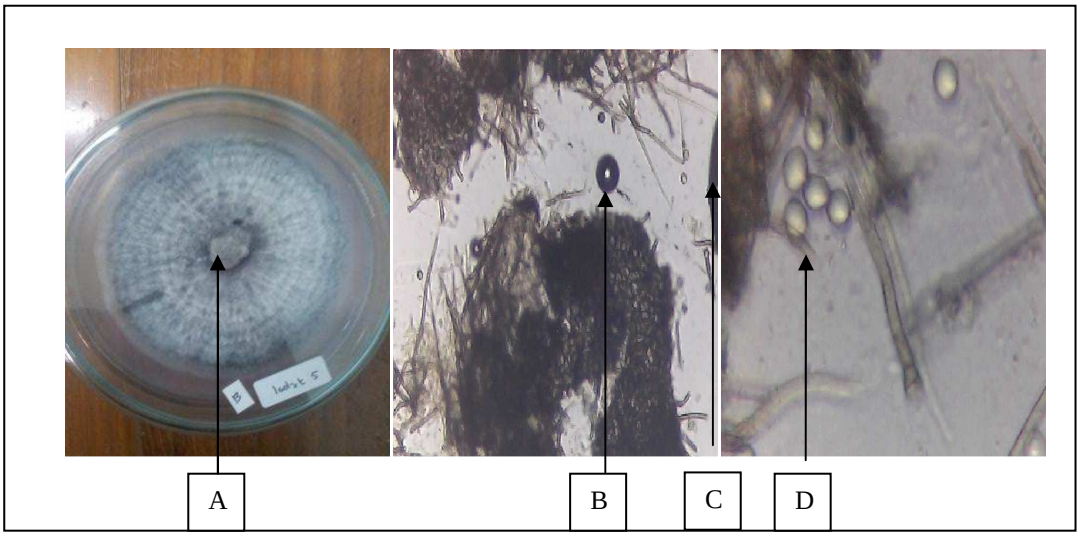

Figure 6. *Aspergillus* sp (a) Colony performance on the PDA (b) Conidiophore (c) Conidia and (d) Hyphae

Penicillium sp

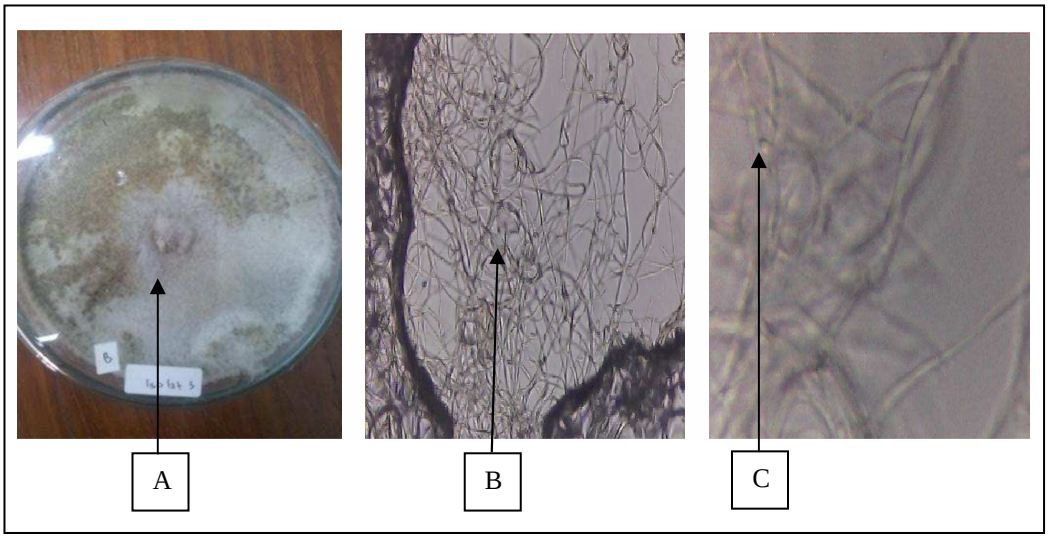

Figure 7. *Aspergillus* sp (a) Colony performance on the PDA (b) Conidiophore (c) Hyphae

Treatment of Rice Black Bugs Cut and Sterilized

The result showed that the treatment rice black bugs cut and sterilized obtained three genera namely *Trichoderma* sp, *Penicillium* sp, *and Aspergillus* sp.(Table 3).

No	Genera	Macroscopic characterization on PDA media	Microscopic characterization
1	*Trichoderma sp*	Green colored colonies and there is also a white spot	- Conidia half-round to oval - Upright branched conidiophores - Conidia at the end fialit
2	*Aspergillus sp*	The Colony is blackish brown	- Smooth Konidiafor somewhat upright - Conidia round - At the end of the round-shaped hyphae
3	*Penicillium sp*	The Colony is white	- Conidiaphore shaped like fingers - There is a branching hyphae 2-3 - Conidia round, slightly oval

Trichoderma sp

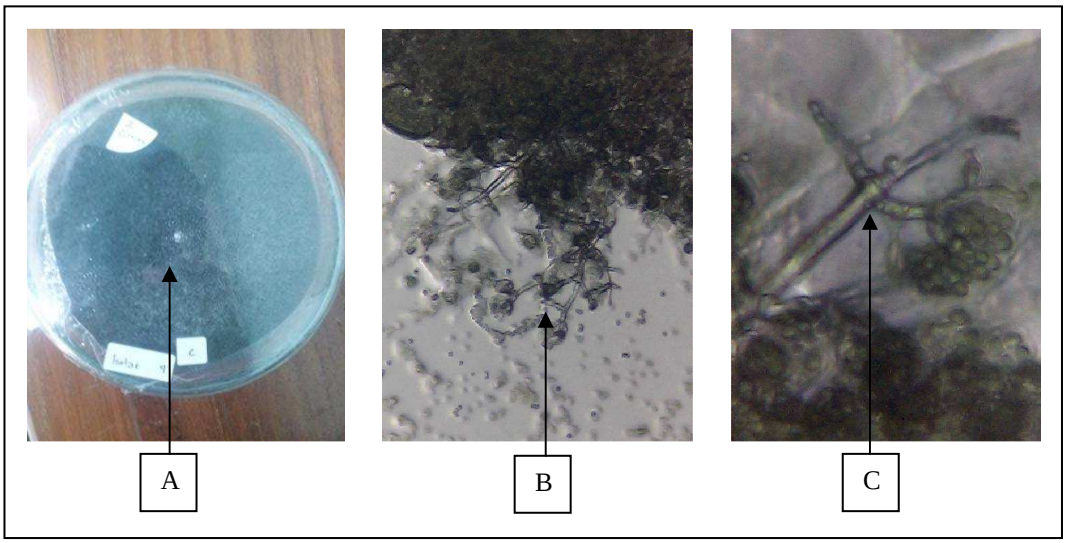

Figure 8. *Trichoderma* sp (a) Colony performance on the PDA (b) Conidiophore (c) Hyphae

Aspergillus sp

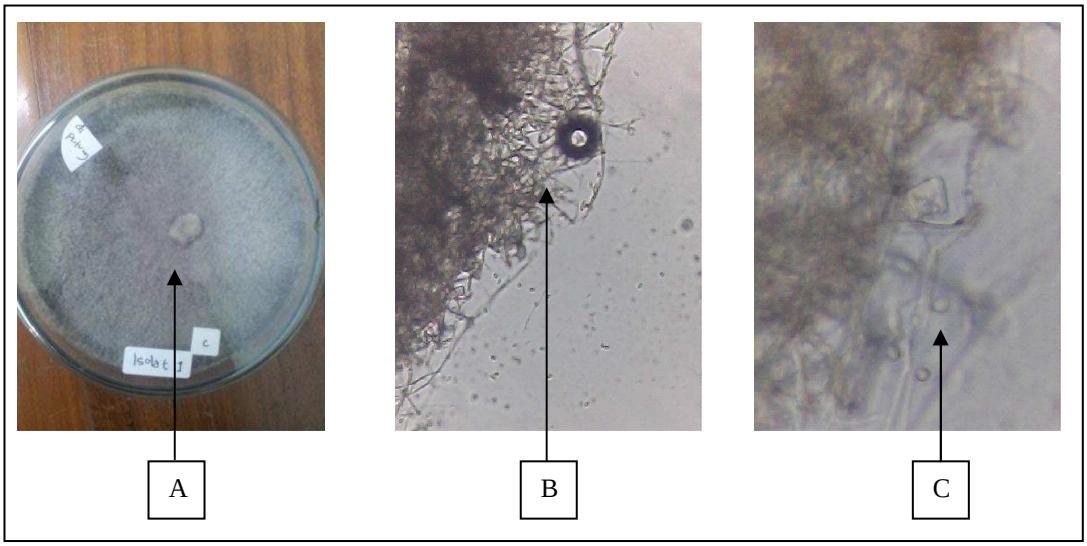

Figure 9. *Aspergillus* sp (a) Colony performance on the PDA (b) Hyphae (c) Conidia

Penicillium sp

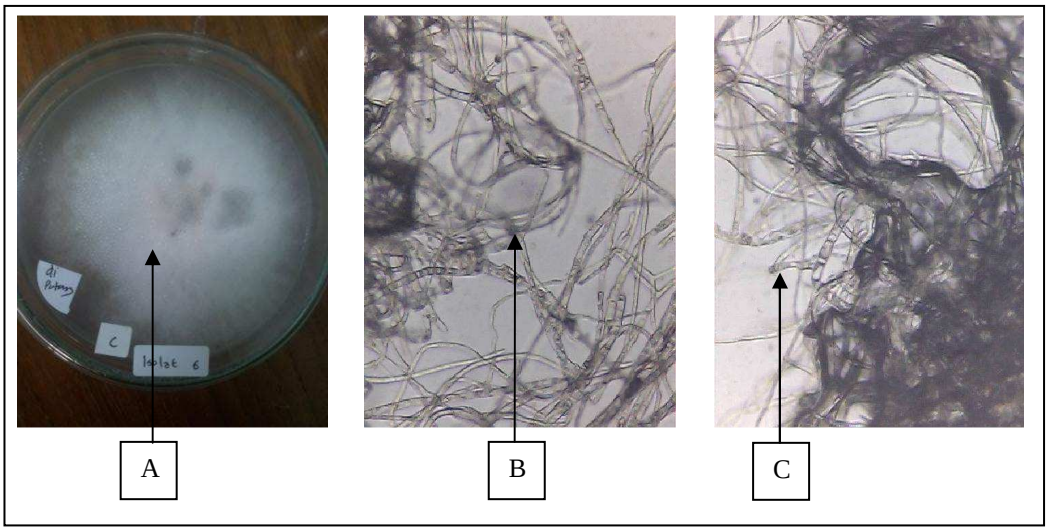

Figure 9. *Penicillium* sp. (a) Colony performance on the PDA (b) Hyphae (c) Conidiophore

DISCUSSION

In the present study by the treatment rice black bug without sterilization obtained three genera namely *Fusarium* sp, *Penicillium* sp, and *Aspergillus* sp. In the treatment of rice black bug with sterilization obtained also three genera of fungi, as well as in the treatment of rice black bugs cut and sterilized, respectively *Metarizium* sp, *Aspergillus* sp, and *Penicillium* sp. as son as *Trichoderma* sp, *Penicillium* sp, and *Aspergillus* sp. In other study in South Sulawesi in addition to *Aspergillus* sp had been isolated from the adult insect rice black bug *Paraeucosmetus pallicornis* [10]. With this phenomenon can be concluded that the possibility of the fungus *Aspergillus* sp. plays an important role so that the rice insect *Paraeucosmetus pallicornis* puncture marks become bitter. More about the role of other fungi obtained in this study is still unclear whether it can be function as entomopathogen.

CONCLUSION

It can be concluded that the treatment rice black bug without sterilization obtained three genera namely *Fusarium* sp, *Penicillium* sp, and *Aspergillus* sp. In the treatment of rice black bug with sterilization obtained also three genera of fungi, as well as in the treatment of rice black bugs cut and sterilized, respectively *Metarizium* sp, *Aspergillus* sp, and *Penicillium* sp. as son as *Trichoderma* sp, *Penicillium* sp, and *Aspergillus* sp.

Acknowledgements
We would like to thank the State Minister of Research and Technology, Republic of Indonesia for the financial support provided for the study, under the Contract of Insentif Riset Sinas 38/SEK/INSINAS/PPK/I/2014. We also expand our thanks to the head of Hasanuddin University Research Institute for his valuable advice during the study.

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